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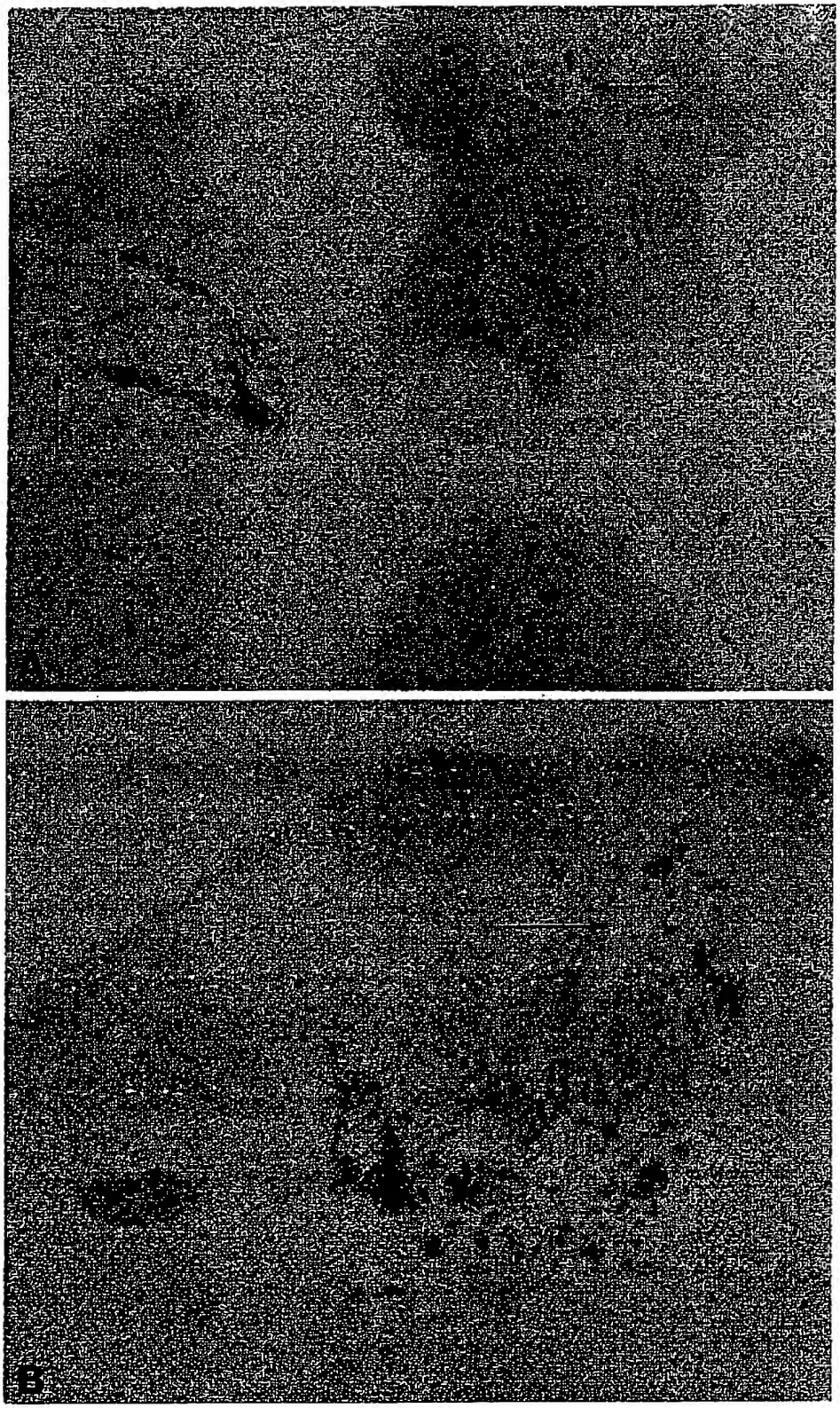

FIG. 3. (A) Detail showing clusters of stained cells, each surrounded by a lucent area of defective myelin (arrows) within lateral and dorsal column white matter. Stained cells are also scattered through gray matter and form linear strings within the central gray and within the lateral white matter at lower left. Same level as Fig. 2B. (B) Detail of Fig. 2F showing clusters of stained cells within central and dorsal horn gray matter and in white matter defects in dorsal and lateral white columns. Arrow indicates one such defect and, beyond it, a stained cell bearing a process.

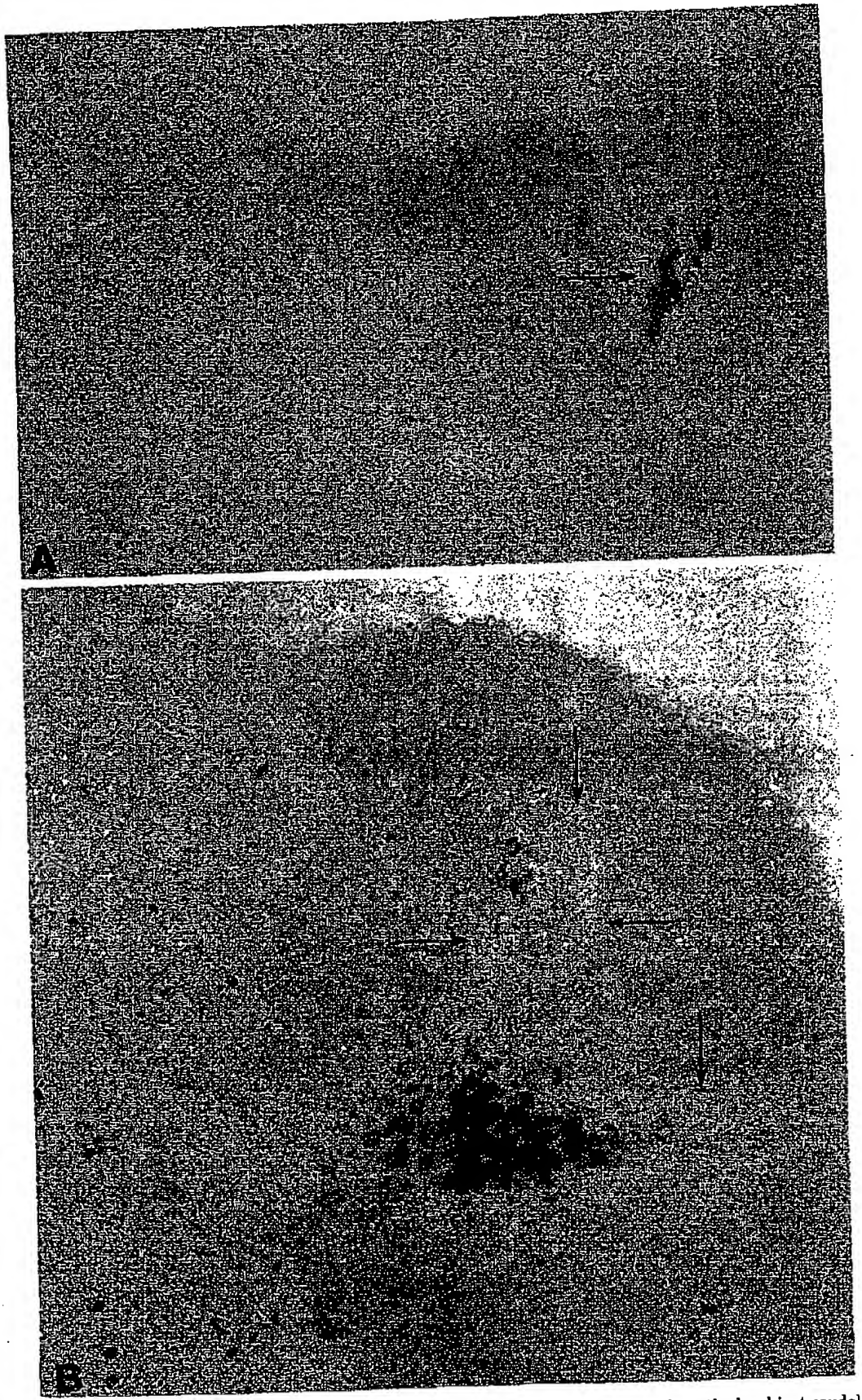

FIG. 4. (A) Stained cells within a lucent area in dorsal column white matter (arrow). Section from the level just caudal to Fig. 2G. (B) Detail of lateral column white matter showing defect (arrows) containing cluster of stained cells. Scattered LacZ$^+$ cells are also present within dorsal gray horn (upper left) and central gray below.

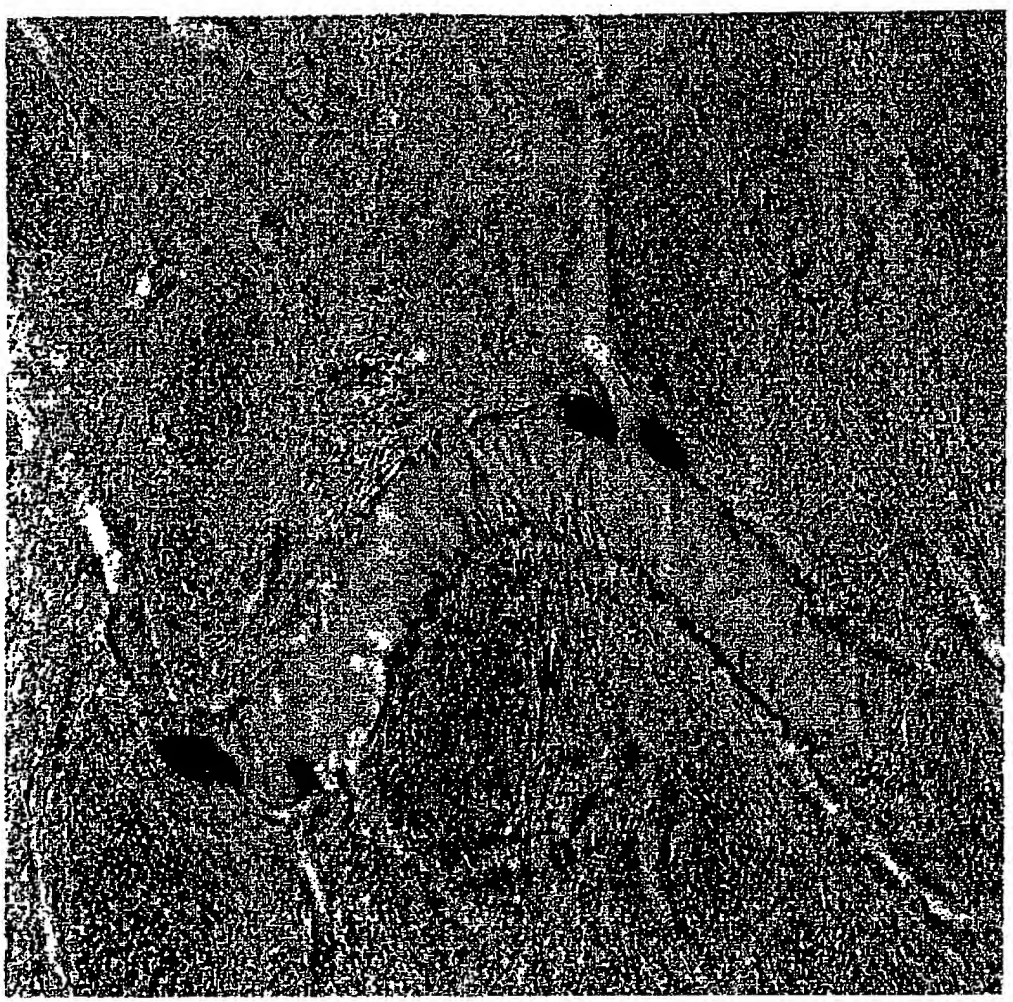

FIG. 5. Brain stem section from same animal as in Fig. 2 showing small dense, strongly LacZ$^+$ cells associated with blood vessel margins.

The length of the processes is consistent with the length of myelin segments. Since the lacZ reaction product is cytoplasmic in location, these stained segments are most simply explained as cytoplasmic components of the myelin sheath, i.e., the external and internal tongues, the paranodal loops, and perhaps other Schmidt–Lanterman-like cytoplasmic channels within the sheath. Such cytoplasmic elements are more conspicuous in newly formed, incompletely compacted myelin segments. Variation in the intensity of the stain could reflect a periodic disposition of these cytoplasmic channels.

Transversely or obliquely oriented processes probably represent the connecting elements that extend between the oligodendrocyte cell body and the outer tongues of the myelin sheaths. They may appear either as extended processes or as stained circular cross sections of small caliber (Figs. 6A and 6B). Such connecting processes are seen as well in transverse sections through fiber tracts, but the multiple elongated processes occur only in the horizontal sections, in which the myelin segments are cut longitudinally.

The second population of LacZ$^+$ cells consists of small dense cells that correspond in morphology and location to the type I oligodendrocytes of Rio Hortega (25). In silver-stained preparations, these give rise to tenuous processes which, however, may be too fine to accommodate the LacZ reaction product and are, therefore, not well demonstrated in our preparations.

In view of the inflammation that follows spinal cord trauma, we also considered the possibility that some of these small dense cells could represent macrophages which engulfed LacZ$^+$ donor cells that did not survive and in which the bacterial enzyme remained sufficiently active to yield reaction product. The size and morphology of these cells is, however, not consistent with that of macrophages laden with cell fragments. Moreover, when sections were immunostained with HAM56, an antibody that recognizes macrophages, the small ovoid LacZ$^+$ cells were HAM56$^-$, while a popula-

G. (B)
within

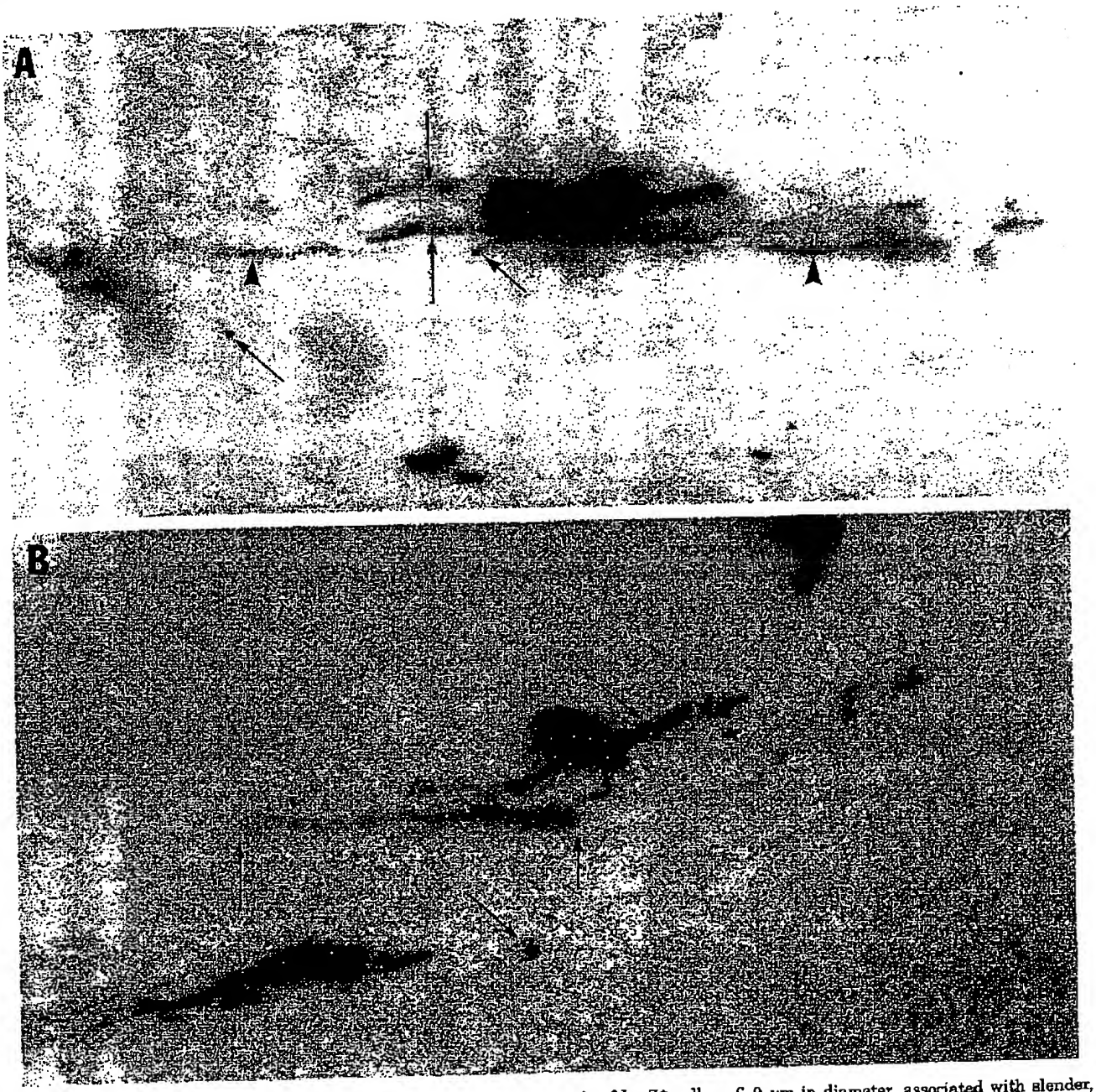

FIG. 6. (A) Horizontal section through dorsal columns showing a pair of LacZ$^+$ cells, ~6–9 μm in diameter, associated with slender, relatively straight longitudinally oriented LacZ$^+$ processes (vertical arrows) measuring ~1–2 μm in diameter and ~30 μm in length, consistent with myelin segments. Two other stained processes (arrowheads) are not clearly connected to these cells. Diagonal arrows indicate obliquely and transversely cut processes. (B) Horizontal section through dorsal columns showing LacZ$^+$ cells, one of which appears to give rise to a longitudinal process ~60 μm long and 2–3 μm wide, which ends abruptly, consistent with a myelin segment. Vertical arrows indicate putative nodes of Ranvier. Periodic densities in the left half of this segment, also visible in A, could represent cytoplasmic expansions. Diagonal arrows show obliquely and transversely cut processes.

tion of LacZ$^-$ cells that had the morphology of macrophages was HAM56$^+$ (D. C. Miller *et al.*, unpublished data). Thus we have no evidence supporting the hypothesis that these LacZ$^+$ cells are macrophages.

Finally, recent studies have shown evidence for apoptosis in endogenous cells of the spinal cord following trauma (13), introducing the possibility that local conditions could induce apoptosis in transplanted cells as well. Thus, some of the small LacZ$^+$ cells lacking apparent processes could be donor glia that differenti-

ated to the point of MBP expression and subsequently underwent apoptosis with accompanying loss of processes. It is of interest, however, that very similar, small LacZ$^+$ cells occur in the adult transgenic mouse spinal cord as well (Fig. 1B) scattered through both gray and white matter and also associated with blood vessels. Since there is no reason to postulate apoptosis in the normal control, cells of this type, in both the normal and traumatized spinal cord, could simply represent the classically described type I oligodendrocytes known to be associated with neurons in gray matter and with blood vessels and which are also present in white matter (25).

The distribution of LacZ$^+$ cells found in the present study (Fig. 2) indicates that the transplanted glia move rostrocaudally over considerable distances. To some extent this movement could be passive, within fluid-filled cystic defects resulting from the trauma. Their presence within intact regions of gray matter, however, where cellular processes are tightly packed, indicates that the cells are also capable of active migration through the tissue. In view of their association with blood vessels, these cells may also move through perivascular spaces, as Vignais *et al.* have proposed (27). The paucity of LacZ$^+$ cells in intact white matter suggests, in contrast, that they avoid migrating into normally myelinated regions or fail to differentiate there.

The fact that LacZ$^+$ cells tend to become concentrated at the defects in myelinated fiber tracts that develop following the trauma could indicate that the transgenic cells move more readily through such defects. Alternatively, the cells may be drawn to such sites chemotactically, or may be retained there, by cytokines, adhesion molecules, or other factors released by the damaged tissue itself or by macrophages that invade secondarily. The cells may also proliferate to a greater extent at these sites than elsewhere or may be induced to mature there to the point of LacZ expression and myelin formation.

Glial transplants from the same transgenic mouse line have been shown previously to form ultrastructurally normal myelin in the myelin-deficient rat spinal cord (21), and it is therefore highly likely that the same cells are capable of myelin formation around rat spinal cord axons in the trauma model as well. The demonstration of 30- to 60-μm LacZ$^+$ segments in horizontal sections supports the conclusion that at least some of the transplanted cells find their way to receptive axons and do in fact form myelin segments.

Transplantation of cultured glial cell suspensions is thus a potentially useful approach to replacing oligodendrocytes in traumatized spinal cord. Such transplants could remyelinate and restore functions lost as a result of demyelination secondary to trauma. Success with xenografted glia suggests, in addition, that the source of the cells transplanted need not be restricted to donors of the same species. Further studies will be required to assess long-term survival of LacZ$^+$ transplanted glial cells and the myelin-like segments produced by them, to determine the optimal parameters for glial transplantation in this model and to determine whether behavioral improvement results from this procedure.

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